METHODS AND MATERIALS

Overview of Study Area and Design

To accomplish the objectives of this study, a "core study" and a "variability study" were designed for each of two field campaigns (Fall 2001 and Spring 2002). These two campaigns collected biota and sediment samples from the four "zones" being modeled by DRBC (Fig. 3). During these two field campaigns, the "core study" centered on the collection of individual fish and prey that were subsequently composited prior to instrumental analysis.

By compositing samples, only mean values were determined. To assess the variability amongst PCB concentrations in white perch and channel catfish, "variability studies" were conducted. To investigate variability in white perch PCB concentrations from fillets only, five individual white perch were analyzed from three separate tows throughout Zone 5 during the fall campaign. Periodic towing throughout the zone ensured a representative population of white perch. In addition to these three separate collections and subsequent individual fillet analyses, composite fillets arising from the 5 adult individuals (>125 mm) from each tow were analyzed.

To investigate variability among catfish, three areas within Zones 2 to 5 were chosen to yield sampling regions harboring different magnitudes of PCB contamination. The three sites (Dredge Harbor in Zone 2, Tinicum Shoals in Zone 4, and just north of Pea Patch Island in the Zone 5) were selected (as outlined in this project's proposal) based on data from two recent studies characterizing concentrations of PCBs in surficial sediment (Costa and Sauer, 1994; Hartwell et al., 2001). Briefly, Tinicum Shoal (PA) was suspected to have higher concentrations of PCBs in surficial sediments, Dredge Harbor (NJ), less so, and finally, just north of Pea Patch Island (DE) sediment was likely to be least compromised by PCB contamination. Catfish variability within these three zones was assessed both in the fall and spring campaigns. At each site, five individual catfish were collected and subsequently analyzed. Additionally, the remaining portions of these individual fillets were composited and analyzed. Prey items and surficial sediments were collected at or near each site of catfish collection.

Sample Collection

Sediment and Fish Collection (Trawl) Locations: The latitude and longitude of each sampling location were recorded using a Trimble Geoexplorer Global Positioning System (GPS) for the fall and spring collections. Additionally, date and time of collection, along with a descriptive sample site id, were recorded. Tables 1 and 2 also denote whether each site was part of the core or variability study (or both). Figures 4 to 7 show the locations of collection of biota and sediment within each zone.

Collected Biota and Sediment Samples: The sample IDs, sex, age, length, and weight of channel catfish and white perch collected from the fall and spring campaigns appear in Tables 3-6. Lists of collected small prey items and invertebrates from the fall and spring campaigns and their

associated sample IDs appear in Tables 7-10. Sediment samples collected from each campaign are tabulate in Tables 11 and 12.

Sediment was collected using CBL's R/V Aquarius using a petite ponar following published guidelines as outlined in the QAPP for this project. Each sample was a composite of three or more separate samples collected within a 5- to 10-m collection radius. Sediment samples consisted of the top 5 cm of the collected sediment. For sediment sample grabs, the sides were removed from each sample so that only mud that had not touched the sampler was used. Sediment samples were transferred to decontaminated stainless steel bowls, homogenized using a decontaminated stainless steel spoon and carefully spooned into pre-cleaned wide mouth glass jars.

All locations of sediment samples were be determined using line of sight with navigational maps, shore-based observations and a laser distance meter or GPS (accurate to < 5 m) as well as ship-board GPS. Criteria for acceptability of grab samples included intact samples with sufficient depth penetration (>10 cm) and a relatively undisturbed sediment surface.

White perch and channel catfish were collected using 20 foot width otter trawls. Upon collection, fish were anaesthetized in Tricaine Methanesulfonate (MS222) and measured for total length. Efforts were made to collect white perch greater than 120 mm TL. Additionally, catfish greater than 300 mm TL were sub-sampled from trawls. Additional samples of white perch and channel catfish beyond the necessary numbers for this study were collected for gut analysis (separate project; Horwitz et al., 2002). Benthic trawls were not successful in Zone 2 due to bottom topography. Samples in this zone were collected using gill netting and/or electroshocking. After on-board measurements were taken, each fish was then be rinsed in D.I. water and wrapped in aluminum foil. Fish were placed on ice on board and at sampling day's end were placed into a freezer for subsequent preparation and chemical analysis.

Prey items (small fish, epibenthic organisms, etc.) were collected using benthic traps, benthic mats and from otter trawls. Upon collection, small fish were anaesthetized in Tricaine Methanesulfonate (MS222) and measured for total length. Subsampled fish were euthanized by overdose in MS222. Each fish was then be rinsed in D.I. water, blotted dry, wrapped in pre-combusted aluminum foil and sealed in a plastic bag. Some samples were placed directly in pre-cleaned jars. Prey items were placed on ice on board and at sampling day's end placed into a freezer for subsequent chemical analysis. Epibenthic organisms, collected from otter trawls were picked from collection nets and using pre-cleaned forceps, counted, sized, and composited in a pre-cleaned glass jar. Benthic organisms were collected using benthic traps and/or benthic mats deployed for numerous days or from sediment grabs. Organisms were allowed to purge their guts prior to freezing. Organisms were identified at the lowest visually identifiable taxa level.

Sample Extraction and Preparation

Sediment and biota samples were frozen and stored below -5°C until extraction. Sub-samples were taken to measure water content. These sub-samples were weighed and allowed to dry at

60°C for 24 hr, cooled to room temperature in a desiccator, and re-weighed to ± 0.001 g. Samples were dried using sodium sulfate. The dried sample was placed in a Soxhlet extracted with dichloromethane (DCM) for at least 18 hr. After extraction, the solvent was reduced in volume, exchanged with hexane using rotoevaporation and further concentrated under a purified N_2 stream. For biota, a fraction of the extracted sample was removed for gravimetric lipid analysis. For sediment analysis, activated elemental copper wool was used to remove elemental sulfur which interferes with the detection of PCB congeners when using an electron capture detector (Ashley and Baker, 1999). Prior to use, the copper was washed by 1 M HCl and rinsed with dichloromethane. The cleaned copper (0.5 - 1 g) was placed in the round bottom flask at the initiation of the Soxhlet extraction step. As a post extraction clean-up procedure, liquid-solid chromatography using florisil was performed on all samples. Florisil was activated at 550 degrees C for 4 hr and deactivated with 2% deionized water prior to use. The deactivated Florisil was placed into a glass column containing a pre-cleaned glass wool plug. Approximately 1 g of precleaned Na_2SO_4 was added on the top. Using this technique, PCBs were eluted from the chromatographic column containing florisil using petroleum ether (F1 fraction). The remaining fraction (F2) was eluted using 50:50 petroleum ether and dichloromethane and archived for subsequent organochlorine pesticide analysis.

PCB Congener Analysis

Congener-specific PCBs were analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 5% phenylmethyl silicon capillary column. The column was 60-m long and had a 0.25-mm internal diameter with 0.25-µm stationary phase film thickness (DB-5, J&W Scientific, Folsom, CA, USA). Hydrogen and argon/methane (ANS) or nitrogen (CBL) were used as the carrier and make-up gases, respectively (flow rates = 30 ml/min), and the inlet pressure was 100 kPa. The temperature program was as follows: 100°C for 2 min, 100-170°C at 4°C/min, 170-280°C at 3°C/min, and 5 min at 280°C. The injector and detector temperatures were 225°C and 285°C, respectively. An auto sampler (HP 7673) was used to inject a 2-µl sample in the splitless injection-mode; data were acquired using both an HP3393A integrator and a computer operating Chemstation software (Hewlett Packard, Palo Alto, CA, USA). The identification and quantification of PCB congeners followed the method of Mullin (1985), in which the identities and concentrations of each congener in a mixed Aroclor standard (25:18:18 mixture of Aroclors 1232, 1248 and 1262) were determined by prior calibration with individual PCB congener standards. Congener identities in the sample extracts were based on their chromatographic retention times relative to the internal standards added. In cases where two or more congeners could not be chromatographically resolved, the combined concentrations were reported. Internal standards were added to all the samples and calibration standards prior to instrumental analysis: 2,3,6-trichlorobiphenyl (congener 30) and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (congener 204).

Otolith Analysis

Age of white perch and channel catfish were determined from analysis of otoliths, the calcium carbonate ‘ear stones’ of these fishes responsible for hearing and equilibrium. Sagittal otoliths were embedded in resin, sectioned in transverse plane, polished with alumina powder, and

viewed under with light microscopy and an imaging system for the presence of annuli. Annuli were enumerated and recorded as annual ages. These analyses were conducted at University of Maryland's Chesapeake Biological Laboratory according to methods described in Secor et al. 1991 (http://cbl.umces.edu/~secor/otolith-manual.html).

Percent Water and Organic Carbon of Sediments

Sediment samples were sub-sampled for measurement of water content. Sub-samples were weighed and allowed to dry at 60°C for 24 hr, cooled to room temperature in a desiccator, and reweighed to ± 0.001g. Sediments were also sub-sampled and analyzed for total organic carbon. Dried and pulverized sediment samples were treated in a desiccator with fuming HCl to remove any inorganic carbon prior to analysis on CE Flash CHN Analyzer. Blanks were analyzed and generally contained carbon near or below the detection limit. Sulfanilamide was used as a primary standard and NIST standard reference materials (SRM 2704 for carbon; SRM 1570a for nitrogen) were used to evaluate analytical accuracy.

Analytical Quality Assurance

Results from all quality assurance and quality control measures are summarized in the QA/QC (Final Report).

Statistical and Mathematical Data Analyses

Routine mathematical analyses of data sets were conducted using Excel. Correlations, analysis of variance and analysis of covariance (ANOVA and ANCOVA were performed using either Excel or the general linear model procedure in SAS or Statistica (at times followed by multiple range tests and planned comparisons of least squares means).

Using SAS, principal component analysis (PCA) was performed on individual PCB congeners to mathematically aid in discrimination of pattern differences/similarities. Our assumption underlying the use of this technique is that samples of common habitat/location use (deemed by collection zone or location within zone) will tend to have similar patterns of PCB congeners, even though absolute concentrations may vary widely due to such factors as age, lipid content and diet. To remove the effect of absolute concentration on the first principal component, individual PCB congener concentrations were normalized to t-PCB (Megan, 1992; Swartz and Stalling, 1991). Congeners that were consistently ranked as non-detect (ND) were not used in this mathematical analysis. In samples where several congener concentrations were below the instrumental detection limit, a value of 0.01 was substituted such that those congeners could be used in the PCA. The first two principal component scores of a PCA were used to detect differences or similarities among individual PCB congener patterns as these often represent the majority of the variance between patterns. The resulting eigenvectors in the principal component equation were used to identify those specific congeners that varied the most between grouping seen in the PCA cross-plot. PCA was only performed using the core data (whole fish). PCA

was initially performed using whole fish from spring and fall; subsequently, fall and spring fish of the same zone and species were averaged to further reduce complexity in the PCA cross-plot.